IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	:	10/809,811	Confirmation No.: 7940
Applicant	:	Nagaraja Rao Ramesh MYSORE et al.	
Filed	:	March 26, 2004	
Title	:	A PROCESS FOR PREPARATION OF FRUCTOOLIGOSACCHARIDES (FOS) POWDER	
TC/AU	:	1657	
Examiner	:	SCHUBERG, Laura J.	
Atty. Docket No.	:	US 1375/04	
Date	:	December 18, 2007	

TRANSMITTAL OF PRIORITY DOCUMENT IN SUPPORT OF CLAIM FOR FOREIGN PRIORITY

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

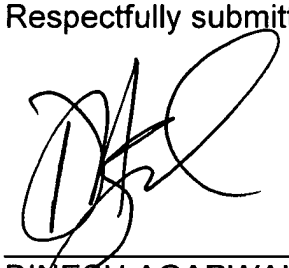
Dear Sir:

Applicants transmit herewith a certified copy of the Indian Patent Application No. 487/DEL/2003, filed March 27, 2003, in support of claim for priority under 35 USC §119.

It is believed that no fee is due for this submission. However, should that determination be incorrect, the Commissioner is hereby authorized to charge any deficiencies, or credit any overpayment, to our Deposit Account No. 01-0433, and notify the undersigned in due course.

Should the Examiner have any questions or wish to discuss further this matter, please contact the undersigned at the telephone number provided below.

Respectfully submitted,



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INTELLECTUAL PROPERTY **INDIA**

सत्यमेव जयते

GOVERNMENT OF INDIA
MINISTRY OF COMMERCE & INDUSTRY
PATENT OFFICE, DELHI
BOUDHIK SAMPADA BHAWAN,
PLOT NO. 32, SECTOR – 14,
NEW DELHI - 110 075.

I, the undersigned being an officer duly authorized in accordance with the provision of the Patent Act, 1970 hereby certify that annexed hereto is the true copy of the ***Application and Complete Specification*** *filed in connection with Patent Application No.* ***487/DEL/2003*** *dated* ***27th March 2003.***

Witness my hand this 7th day of August 2007.

(Dr. Rajesh Dixit)
Assistant Controller of Patents & Designs

FORM 1
THE PATENTS ACT, 1970
(39 OF 1970)
APPLICATION FOR GRANT OF PATENT .
(See Sections 5(2), 7, 54 and 135 and rule 33A)

0487 DEL 03

27 MAR 2003

1. We, COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, Rafi Marg, New Delhi – 110 001, India, an Indian registered body incorporated under the Registration of Societies Act (Act XXI of 1860);

2. hereby declare:

(a) that we are in possession of an invention titled: A process for the preparation of fructooligosaccharides (FOS) using Jaggery

(b) that the ~~Provisional~~ / Complete specification relating to this invention is filed with this application;
(c) that there is no lawful ground of objection to the grant of patent to us;

3. further declare that the inventor(s) for the said invention is / are:
Parinyasakti Thondre Sangeetha, Mysore Nagarajarao Ramesa, Siddalingaiya Gurudutt Prapulla from Central Food Technological Research Institute, Mysore and all are Indian citizens.

DUPLICATE

4. We, claim the priority from the application(s) filed in convention countries, particulars of which are as follows:
NOT APPLICABLE

5. We state that the said invention is an improvement in or modification of the invention, the particulars of which are as follows and of which we are the applicant:
(a) Patent application no.:
(b) Patent application date:

6. We state that the application is divided out of our application, the particulars of which are given below and pray that this application deemed to have been filed on ---------- under section 16 of the Act.
(a) Patent application no.:
(b) Date of filing provisional and / or complete specification ---------- and ----------

7. That we are the assignee of the true and first inventor(s)

8. That our address for service in India is as follows:

Head, IPM Division, CSIR,
INSDOC Building, 14 Satsang Vihar Marg,
New Delhi – 110 067
Phone: 696 2560, 696 8819; Fax : 696 8819

PTO

9. Following declaration was given by the inventor(s):

I / We the true and first inventor(s) for this invention declare that the applicants herein is / are my / our assignee:

Dated this ---27th--- day of ---March---, 2003

Name (in full with expanded initials)

Parigarath Thondre Sangeetha
Mysore Nagaraja Rao Ramesh
S Adalingaiya Gurudutt Kapnle

Signature of the true and first inventor(s)

M. N. Ramesh.

10. That to the best of our knowledge, information and belief the fact and matters stated herein are correct and that there is no lawful ground of objection to the grant of patent to us on this application.

11. Followings are the attachment with the application:

(a) ~~Provisional~~ / Complete specification (3 copies).
(b) Drawings (3 copies).
(c) Priority document(s).
(d) Statement and Undertaking on FORM-3.
(e) Power of authority.
(f) Fee Rs. ---5000/-- in Cheque no.: ---868341--- dated: ---25-3-03--- on State Bank of India, New Delhi Main Branch, Parliament Street, New Delhi – 110 001.

We request that a patent may be granted to us for the said invention.

Dated this ---27th--- day of ---March---, 2003

( )
SCIENTIST
Intellectual Property Management Division,
Council of Scientific and Industrial Research.

डा. श्रीमति इन्द्रा त्रिवेदी
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To,
The Controller of Patents
The Patent Office, New Delhi.

NF 587/02

0487-03

27 MAR 2003

FORM 2

THE PATENTS ACT - 1970
(39 of 1970)

COMPLETE SPECIFICATION

(See Section 10)

A PROCESS FOR THE PREPARATION OF FRUCTOOLIGOSACCHARIDES (FOS) USING JAGGERY

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH,
Rafi Marg, New Delhi - 110 001, India, an Indian Registered Body incorporated under the Registration of Societies Act (XXI of 1860)

The following specification particularly describes the nature of the invention and the manner in which it is to be performed:

The present invention relates to a process for the preparation of fructooligosaccharides (FOS) using jaggery. The process in particular uses the extracellular fructosyl transferase (FTase) from *Aspergillus oryzae* CFR 202.

Fructooligosaccharides are food ingredients that are in great demand for their functional properties. Their non-cariogenicity, low calorific value and prebiotic properties make them suitable for use in beverages, infant milk powders, confectionery, bakery products, yoghurts and dairy desserts.

FOS are usually extracted from plants like Chicory and Jerusalem artichoke or prepared by the action of microbial FTase enzyme on sucrose. Jaggery, a concentrated sugar cane juice with or without purification produced by cottage industries (also known as gur) containing 75 – 85 % sucrose is widely used in India as a substitute for white and refined cane sugar. In the present study, jaggery is used to prepare FOS using FTase from *Aspergillus oryzae* CFR 202.

There are many reports regarding the improvement of the properties and keeping qualities of jaggery and the preparation of various sweet dishes using jaggery. However, there are only very few reports wherein jaggery has been used as an ingredient in the fermentation media.

Reference may be made to S. V. N. Vijayendra, D. Bansal, M. S. Prasad and Krishna Nand, Process Biochemistry, 37, 359-364, 2001, wherein jaggery was used as a novel substrate for pullulan production by *Aureobasidium pullulans* CFR 77. *A pullulans* CFR 77 was grown in batch fermentation using jaggery as a carbon source. The maximum yield of pullulan was obtained using 5 % jaggery in the fermentation broth in 72 h. The process is different from the present process since it results in the production of a polysaccharide.

Reference may be made to P. Ambati and C. Ayyanna, World Journal of Microbiology and Biotechnology, 17, 331-335, 2001 where in palmyra jaggery was used as carbon source for citric acid production using *Aspergillus niger* MTCC 281 by submerged fermentation. Maximum citric acid production was obtained after 136.8 h of fermentation with 221.66 g/L of jaggery in the medium. The process is

different form the present one in that the product is an acid. The drawbacks of the process are the longer fermentation time (136.8 h) and high substrate concentration used in the fermentation medium (221.66 g/L).

Reference may be made to a process for the production of fructooligosaccharides (Prapulla. S. G, Sangeetha. P. T and Ramesh M. N, 439/DEL/2001, March 2001) wherein FOS was prepared using extracellular fructosyl transferase enzyme obtained by growing *Aspergillus oryzae* under submerged fermentation conditions. The process is different form the present one in that it uses only sucrose as the carbon source in the medium and as substrate for FTase to produce FOS.

Reference may be made to an improved process for the production of fructooligosaccharides (Prapulla. S. G, Sangeetha. P. T and Ramesh M. N, 411/DEL/2001, March 2001) wherein FOS was prepared using FTase obtained from the sonicated culture broth of *Aureobasidium pullulans* CFR 77. The process is different from the present one in that it uses the sonicated culture broth as source of FTase and it involves additional steps like sonication and centrifugation to obtain the enzyme. Further, it uses only sucrose as the carbon source for the production of FTase and as substrate for the production of FOS.

The main object of the present invention is to provide a process for the for the preparation of fructooligosaccharides (FOS) using jaggery, which obviates the drawbacks as detailed above.

Accordingly, the present invention provides a process for the preparation of fructooligosaccharides (FOS) using jaggery, which comprises

(a) growing the culture *Aspergillus oryzae* CFR 202 in a medium consisting of 1 % sucrose and 0.2 % yeast extract (pH 5.5) at 25 - 30 ^{0}C at 200 - 250 rpm for 24 - 48 h to develop the inoculum
(b) transferring 20 % of the inoculum to a medium consisting of 10 % sucrose / jaggery, 0.8 % yeast extract, 0.03 % $MgSO_4.7H_2O$, 2 % $NaNO_3$, 0.4 % K_2HPO_4, 0.9 % KH_2PO_4, 0.6 % NaCl and 1 % NH_4Cl and growing for a period of 48 - 120 h under submerged fermentation conditions

(c) separating the pellets from culture fluid by filtration using filter paper

(d) incubating the culture fluid with the substrate for 18-24 h at 50 - 55 ^{0}C, pH 5.0 – 5.5 and

(e) analyzing the reaction mixture for the content of FOS, which yielded up to 48 %.

In an embodiment of the present invention, the culture used may be from *Aspergillus oryzae*.

In another embodiment of the present invention, the inoculum used may be developed from 5-8 days old slant culture.

In yet another embodiment of the present invention, the culture fluid may be incubated with sucrose / jaggery as substrate in a concentration of 400 g/L to 800 g/L.

The substrate solution was prepared by weighing sucrose / jaggery (60 g) and dissolving it slowly in 0.1 M citrate buffer (pH 5.0) and making up the total volume to 100 ml using citrate buffer.

The process for the preparation of fructooligosaccahrides using jaggery is illustrated in the following flow chart

Five –Eight days old slant of *Aspergillus oryzae* CFR 202

↓

One loop full of spores transferred to medium containing 1 % sucrose / jaggery and 0.2 % yeast extract at pH ranging from 5-6 and incubated for 24-48 h at temperature ranging from 25-30 ^{0}C at about 200-250 rpm to develop inoculum

↓

20 % inoculum transferred

↓

Fermentation medium consisting of 10 % sucrose / jaggery, 0.8 % yeast extract, 0.03 % $MgSO_4.7H_2O$, 2 % $NaNO_3$, 0.4 % K_2HPO_4, 0.9 % KH_2PO_4, 0.6 % NaCl and 1 % NH_4Cl and incubated for 48-120 h at temperature ranging from 25-30 ^{0}C

↓

Filtered the culture broth

↓ ——→ Pellets discarded

Fructosyl Transferase (FTase)

↓

FTase (0.25 ml) mixed with 600 g/l sucrose / jaggery(1.75 ml) at pH 5.0-5.5 for 18-24 h at 50-55^{0}C

↓

Fructooligosaccharides

The novelty of the process is that it uses jaggery as carbon source in the fermentation medium and as substrate to prepare FOS. The final product contains 48.59 % FOS with functional properties like non-cariogenicity, low calorific value and prebiotic property. FOS also improves mineral absorption, reduces the total cholesterol and triglyceride levels in the body. Another novelty of the process is the value addition to a lower value gur to a high value product with FOS

The following examples are given by way of illustration of the present invention and therefore should not be constructed to limit the scope of the present invention.

EXAMPLE – 1

Aspergillus oryzae was grown in 50 ml medium consisting of 1 % sucrose and 0.2 % yeast extract (pH 5.5) at 30 ± 1 ^{0}C for 24 h at 250 rpm to develop inoculum. 20 % v/v of the inoculum was transferred to 50 ml fermentation medium in a 250 ml conical flask containing 10 % sucrose, 0.8 % yeast extract, 0.03 % $MgSO_4.7H_2O$, 2 % $NaNO_3$, 0.4 % K_2HPO_4, 0.9 % KH_2PO_4, 0.6 % NaCl and 1 % NH_4Cl and incubated at 250 rpm for 90 h at 30 ± 1 ^{0}C. The pellets were separated from the culture fluid by filtration using filter paper (Whatman No. 2). The culture fluid obtained was used as the source of extracellular enzyme for the production of FOS. 0.25 ml of the culture fluid was mixed with 1.75 ml of the substrate (600 g/L jaggery) and incubated for 18 h at 55 ^{0}C at pH 5.15. The reaction was stopped by keeping the reaction mixture in boiling water bath. The reaction products were analyzed by HPLC using refractive index detector. The maximum FOS yield obtained was 41.98 % of the initial sucrose which consisted of 32.07 % Kestose and 9.91 % Nystose.

EXAMPLE – 2

Aspergillus oryzae was grown in 50 ml medium consisting of 1 % sucrose and 0.2 % yeast extract (pH 5.5) at 30 ± 1 ^{0}C for 24 h at 250 rpm to develop inoculum. 20 % v/v of the inoculum was transferred to 50 ml fermentation medium in a 250 ml conical flask containing 10 % jaggery, 0.8 % yeast extract, 0.03 % $MgSO_4.7H_2O$, 2 % $NaNO_3$, 0.4 % K_2HPO_4, 0.9 % KH_2PO_4, 0.6 % NaCl and 1 % NH_4Cl and incubated at 250 rpm for 90 h at 30 ± 1 ^{0}C. The pellets were separated from the culture fluid by

filtration using filter paper (Whatman No. 2). The culture fluid obtained was used as the source of extracellular enzyme for the production of FOS. 0.25 ml of the culture fluid was mixed with 1.75 ml of the substrate (600 g/L jaggery) and incubated for 18 h at 55 ^{0}C at pH 5.15. The reaction was stopped by keeping the reaction mixture in boiling water bath. The reaction products were analyzed by HPLC using refractive index detector. The maximum FOS yield obtained was 40.03 % of the initial sucrose which consisted of 32.75 % Kestose and 7.28 % Nystose.

EXAMPLE – 3

Aspergillus oryzae was grown in 50 ml medium consisting of 1 % sucrose and 0.2 % yeast extract (pH 5.5) at 30 ± 1 ^{0}C for 24 h at 250 rpm to develop inoculum. 20 % v/v of the inoculum was transferred to 50 ml fermentation medium in a 250 ml conical flask containing 10 % jaggery, 0.8 % yeast extract, 0.03 % $MgSO_4.7H_2O$, 2 % $NaNO_3$, 0.4 % K_2HPO_4, 0.9 % KH_2PO_4, 0.6 % NaCl and 1 % NH_4Cl and incubated at 250 rpm for 90 h at 30 ± 1 ^{0}C. The pellets were separated from the culture fluid by filtration using filter paper (Whatman No. 2). The culture fluid obtained was used as the source of extracellular enzyme for the production of FOS. 0.25 ml of the culture fluid was mixed with 1.75 ml of the substrate (600 g/L sucrose) and incubated for 18 h at 55 ^{0}C at pH 5.15. The reaction was stopped by keeping the reaction mixture in boiling water bath. The reaction products were analyzed by HPLC using refractive index detector. The maximum FOS yield obtained was 48.59 % of the initial sucrose which consisted of 38.47 % Kestose and 10.12 % Nystose.

EXAMPLE – 4

Aspergillus oryzae was grown in 50 ml medium consisting of 1 % sucrose and 0.2 % yeast extract (pH 5.5) at 30 ± 1 ^{0}C for 24 h at 250 rpm to develop inoculum. 20 % v/v of the inoculum was transferred to 50 ml fermentation medium in a 250 ml conical flask containing 10 % sucrose, 0.8 % yeast extract, 0.03 % $MgSO_4.7H_2O$, 2 % $NaNO_3$, 0.4 % K_2HPO_4, 0.9 % KH_2PO_4, 0.6 % NaCl and 1 % NH_4Cl and incubated at 250 rpm for 90 h at 30 ± 1 ^{0}C. The pellets were separated from the culture fluid by filtration using filter paper (Whatman No. 2). The culture fluid obtained was used as the source of extracellular enzyme for the production of FOS. 0.25 ml of the culture

fluid was mixed with 1.75 ml of the substrate (600 g/L sucrose) and incubated for 18 h at 55 ^{0}C at pH 5.15. The reaction was stopped by keeping the reaction mixture in boiling water bath. The reaction products were analyzed by HPLC using refractive index detector. The maximum FOS yield obtained was 58 % of the initial sucrose which consisted of 32.62 % Kestose, 22.47 % Nystose and 3.06 % Fructofuranosyl nystose.

A summary of the results given in the examples are presented in Table 1

Table 1

Serial no.	Carbon source in the media	Substrate	% FOS
1	Sucrose	Jaggery	41.98
2	Jaggery	Jaggery	40.03
3	Jaggery	Sucrose	48.59
4	Sucrose	Sucrose	58.00

The inferences from the above examples are detailed below.

1. FTase can be obtained from *A oryzae* CFR 202 using jaggery / sucrose as carbon source in the fermentation medium.
2. FOS can be obtained by incubating extracellular FTase from *A oryzae* CFR 202 with jaggery / sucrose.
3. Fermentation time required for producing the enzyme, fructosyl transferase can be 48-96 h.
4. The yield of FOS obtained is 40 % using jaggery as carbon source in fermentation media and substrate, 41.98 % using sucrose in fermentation media and jaggery as substrate and 48.59 % using jaggery in fermentation media and sucrose as substrate where as the FOS yields obtained using sucrose in fermentation media and substrate is 58 %.

The advantages of the present invention are:

1. FTase obtained by growing *A oryzae* CFR 202 on a cheaper carbon source like jaggery.
2. FOS can be obtained using jaggery / sucrose as substrate at a concentration of 600 g/L.
3. A minimum of 90 h of fermentation is only needed to produce higher titres of fructosyl transferase.

We claim

1. A process for the preparation of fructooligosaccharides (FOS) using jaggery, which comprises;

   (a) growing the culture in a medium consisting of 1 % sucrose and 0.2 % yeast extract (pH 5.5) at 25-30 ^{0}C at 200-250 rpm for 24-48 h to develop the inoculum

   (b) transferring 20 % of the inoculum to a medium consisting of 10 % sucrose / jaggery, 0.5 % yeast extract, 0.05 % $MgSO_4.7H_2O$, 1 % $NaNO_3$, 0.25 % K_2HPO_4, 0.25 % KH_2PO_4, 0.25 % NaCl and 0.5 % NH_4Cl and growing for a period of 48 - 120 h under submerged fermentation conditions

   (c) separating the pellets from culture fluid by filtration using filter paper

   (d) incubating the culture fluid with the substrate for 18-24 h at 50-55 ^{0}C, pH 5-5.5

   (e) analyzing the reaction mixture for the content of FOS, which yielded up to 48.59 % of the initial sucrose concentration.

2. A process for the production of fructooligosaccharides as claimed in claim 1 wherein the culture used is *Aspergillus oryzae*.

3. A process for the production of fructooligosaccharides as claimed in claims 1 & 2 wherein the inoculum used is developed from 5 -8 days old slant culture

4. A process for the production of fructooligosaccharides as claimed in claims 1 to 3 wherein the culture fluid is incubated with sucrose / jaggery as substrate in a concentration of 400 g/L to 800 g/L.

5. A process for the production of fructooligosaccharides substantially as herein described with reference to the examples accompanying this specification.

Dated this **27th** day of **March** 2003

( )

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Intellectual Property Management Division

Council of Scientific & Industrial Research

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